Amendments to the Specification:

Please replace the original Abstract as filed with the amended Abstract which is found at the end of this paper on a separate sheet as required.

Please replace 4th paragraph of page 2 with the following amended paragraph:

However, both the steps of exchanging protein from the column by a decoupling reagent and the biotinylation of the protein need to remove the decoupling reagent and unreacted reagents by dialysis or molecular sieve purification. These are not ideal procedures. Dialysis has three major ~~shortcomes~~ shortcomings : (1) inconvenient: large amount of dialytic solution needs to be prepared, and the work becomes tedious when multiple samples are to be dialyzed at the same time; (2) time-consuming: it requires at least 2-3 times of balance, each takes at least 4-6 hours, and therefore it takes at least one day to complete a dialysis; (3) low recovery of protein: protein may denature or attach to the dialysis membrane. Molecular sieve purification has two main problems: (1) small ~~volumn~~ volume of the column: only one-tenth column ~~volumn~~ volume of sample can be processed in the molecular sieve column each time; (2) low recovery and dilution: due to changes of salt concentration within the column during the elution process, protein will ~~attaché~~ attach to the column and diluted by the elution solution. Therefore, it is an important issue to improve protein recovery rate during the purification and modification steps.

On page 4, please replace the fifth full paragraph with the following amended paragraph.

The metal chelation column is represented by a general formula as metal-X column. The "metal" in said formula used herein refers to the metal having affinity attraction with histidine tag, said metal comprises, but not limited to, nickel(Ni), zinc(Zn),

cobalt(Co), or copper(Cu). The "X" in said formula used herein refers to the matter that can chelate with the aforementioned metal, said X comprises, but not limited to, iminodiacetic acid(IDA) nitrilotriacetic acid(NTA), tris(carboxymethyl)ethylendiamin, carboxymethylaspartate, or TALON. TALON is a trademark resin used in IMAC (immobilized metal affinity chromatography). Specifically, it is carboxymethylated aspartate ligand complexed with a transition metal ion in a 2.sup.+ oxidation state, having a coordination number of 6.

Please replace 2nd paragraph of page 5 with the following amended paragraph:

The immobilization of the recombinant protein is based on the binding between the coating of substrate and the modifying functional group added to the recombinant protein. When the recombinant protein is carries a biotin functional group, the coating of substrate is streptavidin. The substrate comprises metal (e.g. iron beads), glass or polymers.

Please replace 2nd paragraph of page 7 with the following amended paragraph:

The modification of the recombinant protein means adding a molecule to the recombinant protein. The molecule has specific affinity with another molecule. An example is biotin and streptavidin. Biotin can be added to many kinds of biomolecules, including DNA and protein. The specific and strong binding between biotin and streptavidin has been increasingly applied to molecular biology ~~researchs~~ research.

Please replace 1st paragraph of page 8 with the following amended paragraph:

Example 1 is a method for purifying, modifying and immobilizing recombinant protein of the present invention according to the flow chart shown in FIG. 2. *E. coli*

BL21De3 was used as the expression host for the pET28A (Novagen) vector containing the recombinant gene. Incubating with LB media containing the inducer, IPTG (1mM), *E. coli* expressed the designed recombinant thrombospondin N-terminal like domain (TSPN like domain) of human collagen type 21 containing a Histidine tag. As shown in FIG. 3, comparing with Lane 2 (without IPTG induction), Lane 1 (with IPTG induction) showed the recombinant TSPN like domain ~~at~~ where the arrow is pointing. Lane 3 and Lane 4 were the molecular weight markers. Lane 5 showed the primarily purified recombinant TSPN like domain. Its molecular weight is 25 kD, which conformed the expected total molecular weight of TSPN like domain and Histidine tag. The recombinant TSPN like domain solution was then passed through a Ni-IDA affinity chromotography column. The recombinant TSPN like domain having the Histidine tag was selectively retained in the column. The solution passing out the column contained almost no recombinant TSPN like domain (Lane 6). Biotinylation reagent, NHS-LC-biotin, was then added to modify the recombinant TSPN like domain in the column so to attach biotin to the protein. This reaction would not effect the specific attachment of the recombinant TSPN like domain to the affinity column, and therefore the solution passing out the column did not contain the recombinant TSPN like domain (Lane 7). After the reaction was completed, the column was washed with buffer to remove unreacted biotinylation reagent. The buffer passing out the column also did not contain the recombinant TSPN like domain (Lane 8). Next, a decoupling reagent (Imidazole) was used to exchange the recombinant TSPN like domain from the affinity column. Since the recombinant TSPN like domain now carried the biotin, the molecular weight had increased (Lane 9).~~Mixed the recombinant TSPN like domain modified with biotin and iron beads modified with streptavidin~~ <u>The recombinant TSPN like domain modified with biotin was mixed with iron beads modified with streptavidin</u>. When biotin binded tightly to streptavidin, the iron beads were removed from the solution by a magnet. The solution was now free of the recombinant TSPN like domain (Lane 10).